Sarnoff Corporation

MicroLab: a High-Throughput, Low-Cost Approach to DNA Diagnostics

In 1994, molecular analysis of DNA was a nascent technology. The David Sarnoff Research Center was known for creating and introducing breakthrough new electronics technologies, and researchers wanted to enter the biotechnology arena. Sarnoff applied to ATP and proposed to combine the principles of biochemistry and electronics to create a "MicroLab," which could analyze DNA samples at high speed and low cost. The MicroLab would make available automated, user-friendly DNA diagnostics in the doctor's office for more directed and faster medical treatments. The MicroLab would consist of a 3-inch-square piece of glass that would contain microscopic test tubes and hair-thin channels to facilitate 10,000 simultaneous experiments. The multidisciplinary study of fluid behavior at volumes thousands of times smaller than a common droplet is called microfluidics. The MicroLab system would integrate state-of-the-art computer hardware and software for data collection and reporting. The goal was to increase healthcare effectiveness and reduce cost by providing clinics and other healthcare providers with a fast, accurate, and cost-effective instrument to perform DNA analyses. The MicroLab could also be used in drug development to synthesize new molecules and test them against disease, without human intervention or contamination.

The Advanced Technology Program (ATP) awarded Sarnoff cost-shared funding as part of a focused program, "Tools for DNA Diagnostics," for a three-year project that began in 1995. This project was the genesis of Sarnoff's biotechnology efforts and would not have been possible without ATP funding. Sarnoff started a new company, Orchid BioComputer, in 1995 based on Microlab technology. By 1998 Sarnoff developed a prototype MicroLab, was awarded 12 patents for their innovations in miniaturization, and published its results. Sarnoff passed these achievements to Orchid. Orchid became a public company and was renamed Orchid BioSciences in 2000. Sarnoff continued to develop microfluidics and spun off another new company, Rosedale Medical, in 2003, which is currently developing glucose-monitoring equipment for diabetics. Although Orchid did not directly commercialize MicroLab, the company continued enhancing microfluidics development and built on the core knowledge they gained in this ATP-funded project to develop related DNA diagnostics services. Orchid began providing DNA analyses on a fee-for-service basis in 2001 and became a leading provider of identity genomics services for the forensic and paternity DNA testing markets.

COMPOSITE PERFORMANCE SCORE
(based on a four star rating)
* * *

Research and data for Status Report 94-05-0029 were collected during November – December 2004.

Emerging DNA Industry Lacks Focus

In DNA sequencing or decoding, scientists analyze a long string of DNA and identify its fragments to determine information relating to disease. Traditional molecular biology methods relied on a manual "one-gene-in-one-experiment" basis to analyze DNA. This process was time consuming and made it difficult to get a complete picture of an individual's health issues. These manual, repetitive processes were also hampered by frequent contamination problems. In 1994, analysts predicted that faster, broader, and less

costly automated DNA analysis techniques could be developed, but paths to funding and market dynamics for the new industry were unclear. Moreover, developers faced an uncertain future. Clinics, doctors and drug companies were reluctant to invest in technology that was unproven in the marketplace.

Patients and doctors would benefit from small, low-cost, automated DNA diagnostics tools. Potential medical benefits of such a tool included the following:

- Increased accuracy and speed of diagnosis and, thus, treatment
- Improved discovery for new drug therapies, leading to novel disease treatment options

While the David Sarnoff Research Center had no prior experience with biotechnology, they did have strong experience in electronics. Sarnoff researchers believed that their strength in miniaturization of electronics, combined with their understanding of the problems in DNA analysis tools and the specialized expertise of several new staff members, could lead to dramatic progress in DNA analysis technology.

Sarnoff Proposes a "MicroLab" DNA Analysis Tool

In 1994, Sarnoff proposed to develop the key technologies for the "MicroLab," an automated instrument consisting of miniaturized devices in which microvolume samples of blood would be analyzed for infectious and genetic diseases. The system would run numerous reactions of moderate complexity in parallel. MicroLab's innovative design would consist of glass "chips," approximately 2.5 inches square, with an array of DNA probes. (Arrays are DNA sequences laid down on the chip to create an orderly arrangement of probes for testing.)

Traditional molecular biology methods relied on a manual "one-gene-in-one-experiment" basis to analyze DNA.

Each MicroLab would serve as an individual, specialized chemical laboratory for DNA diagnostic procedures. Ten thousand chemical experiments could be conducted simultaneously on the glass chip using microscopic test tubes connected by hair-thin channels and reservoirs that are computer-controlled by millimeter-scale electronic pumps, valves, and chemical sensors. The full system would include data collection, processing, and reporting functions based on state-of-the-art computer hardware and software technology. If successful, the MicroLab would give clinics a fast, accurate, cost-effective, stand-alone instrument for DNA analyses. It could also be used in drug development: developers could synthesize molecules automatically to test them against diseased cells, without the risk of contamination or high labor costs.

Sarnoff planned to rely on several subcontractors: Nalge Nunc International to develop microvalves and syringes; Dynal Direct to develop reagents; and Rela, Inc. to produce instrumentation to control the system.

Researchers had to address the following technical risks:

- Scaling down the chemistry of DNA assays, transferring reagents to minute channels, and sterilizing the surfaces. Actual effective fluid flow in channels was believed to fail at channel widths of one micrometer (a micrometer is 1/1000th of a millimeter). Researchers planned to use channels of 10 micrometers or greater.
- Precisely measuring, dispensing, and transferring various fluid volumes, controlling multiple fluid flows, and reliably detecting microvolumes.
- Addressing system issues (such as electrical control of heaters), data collection, and system and patient identification.
- Preparing samples for microvolume processing, especially separating white blood cells from whole blood.
- Controlling background DNA (impurities) prior to making multiple copies of the desired DNA for analysis.

Because of the high technical risk, Sarnoff was unable to find commercial investing partners or venture capital

investors for this project. ATP awarded cost-shared funding to Sarnoff as part of a focused program, “Tools for DNA Diagnostics,” beginning in 1995.

Automated MicroLab Would Offer Low-cost DNA Analysis

RNA indicates which genes are expressed in a tissue. Expressed genes tell the cells which functions to perform. For example, the same DNA molecules are in every cell, but liver or kidney tissues express, or “turn on” different genes. A cell from a diseased tissue sample may under- or over-express a particular gene. For example, genes that affect blood diseases are expressed in the bone marrow, which produces blood; these genes would not be expressed in the kidney or liver. In the case of sickle cell anemia, the gene that instructs cells to produce hemoglobin is improperly coded, and in hemophilia, the gene for a blood-clotting factor is missing. Sarnoff’s vision was to develop an integrated, automated, low-cost miniaturized MicroLab on a chip that would include three modules for sample preparation, amplification, and detection of expressed genes in a sample, as described below.

- *Sample preparation* (preparing a representative sample, with the appropriate RNA). Sarnoff would homogenize tissue and isolate RNA from the cells while avoiding contamination. The isolated RNA is then reverse transcribed to generate copies of DNA (an enzyme step to synthesize DNA using RNA as a template).

- *Amplification* (polymerase chain reaction [PCR]). PCR is a method of replicating numerous copies of DNA required prior to analysis.

- *Detection* (complementary DNA [cDNA] creation and hybridization). cDNA represents only the expressed genes. MicroLab “labeled” them by attaching a fluorescent dye that makes them detectable. MicroLab measured the fluorescent signal at specific probe locations to measure the expressed genes. This was the final step in the DNA analysis.

While research and development were in the early stages, Sarnoff used the concepts for the ATP-funded project and collaborated with SmithKline Beecham (name changed to Glaxo SmithKline) to form Orchid BioComputer in 1995 in order to pursue drug discovery. Speaking about MicroLab, Dr. Carmen Catanese, Vice President of the Electronic Systems Division at Sarnoff, said, “We see this as a revolution in the drug-discovery process, merging biochemistry and electronics, and Sarnoff is excited to be teaming with SmithKline Beecham in making it happen. The result will be quicker discovery cycles for pharmaceuticals and will enable companies like [SmithKline Beecham] to be in the forefront of drug discovery.” SmithKline Beecham, which discovers, develops, manufactures, and markets human pharmaceuticals and health-related consumer products, primarily played the role of customer, with an eye toward future commercialization. This project was later discontinued, because the path to drug molecule testing and discovery was not yet commercially viable. However, the core technology from this project enabled the start-up company, Orchid, to thrive commercially based on other technology it purchased.

Sarnoff’s business model relied on starting up new companies to commercialize technologies. "What we do with these start-up companies," said Anne Van Lent, Sarnoff’s vice president of ventures, "is contribute our patents and intellectual property, we license them into the company on an exclusive basis in exchange for our piece of the equity, then we go out and solicit funding from the investment community. They become free-standing corporations in which Sarnoff just has an equity interest." Sarnoff continued to pass its ATP-funded knowledge and patents to Orchid through the duration of the project.

“We see this as a revolution in the drug-discovery process, merging biochemistry and electronics…”

Sarnoff initiated an additional new program in biotechnology in 1996, after the ATP project was well into its first year. The new project was a clear extension of the concepts of the ATP-funded project. Under this project, funded by the Defense Advanced Research Projects Agency (DARPA), Sarnoff developed the Autonomous Microfluidic Detection System (AMDS). AMDS was based on multiple integrated chemistries performed in silicon or glass chip structures for a miniaturized system. The AMDS would make the same detections as those made by existing large-scale,

multiple instruments in biochemical labs, only using smaller quantities of chemical reagents. Sarnoff also transferred the AMDS development to Orchid. Sarnoff researchers believed that the concepts researched under both the ATP and DARPA projects could be applied to new drug discovery as well as high-speed, low-cost DNA analysis.

Sarnoff Completes a Prototype MicroLab

By 1998, at the conclusion of the ATP-funded project, Sarnoff researchers had realized 90 percent of their technical goals in a prototype MicroLab, which focused on the fundamentals of miniaturization. The advances for all three modules are described below.

- *Sample preparation*. Sarnoff integrated unique devices and instruments. They transferred fluids with a fabricated valve, fully automated the system, and used custom syringes to provide precision transfer. They developed a novel closed, contained, disposable cassette that permitted contaminant-free sample processing. The team reduced sample and reagent volumes from 100 microliters to 1 microliter. This slashed consumption and, therefore, the cost of chemicals used in sample preparation.

- *Amplification*. The team designed and fabricated a disposable cassette to contain the sample for PCR. They also assembled and validated a fast thermal-cycling process, synthesized pure PCR product, and developed new methods for automated in-vitro amplification.

- *Detection*. The team validated highly sensitive membrane-enhanced fluorescent detection. They reduced total analysis time required from 24 hours to 3 hours and reduced DNA amplification and detection time from 90 minutes to 20 minutes. The reduced labor requirements of the automated analysis system facilitated high throughput sample screening (large numbers of samples). Data indicated that nylon membrane substrates have binding and fluorescence properties advantageous to small-volume assays. The team began discussions with Pall Corporation (East Hills, NY) to form a joint venture to develop and apply the membrane-enhanced fluorescence.

Sarnoff received 12 patents for these innovations. MicroLab technology dramatically reduced cost and enabled diagnostics in new ways. Moreover, data showed the superior quality of the DNA sample preparation. The team validated the MicroLab prototype against strict performance expectations; however, some technical issues remained, such as with the sealing fluid in PCR amplification and storing reagents.

Sarnoff's MicroLab development was the company's first entry into the biotechnology arena, which they could not have done without ATP support. Sarnoff researchers still predict that, over the next 20 to 30 years, DNA analysis tools like MicroLab will move to hospitals and doctors' offices to facilitate fast, accurate diagnoses. In the short term, MicroLab was not commercialized, because of a lack of outside financial support. Neither health insurance companies nor healthcare providers were willing to incur the expense of purchasing the equipment. However, as consumers learn more about the benefits of DNA analyses, demand is expected to grow, which will push providers to find ways to overcome current funding barriers. Microfluidics will continue to evolve, along with supporting technologies that include the machinery needed prior to analysis.

Microfluidics Serve Diverse Applications

Sarnoff continues to develop microfluidic applications based on its ATP-funded technology. Company researchers had developed comprehensive skills in microfluidics and medical devices and, in 2003, completed a feasibility study for a glucose-monitoring device. Despite difficult economic times, they successfully started up another new company in 2003, Rosedale Medical, Inc. Rosedale is developing instruments to automate and simplify glucose monitoring for diabetics. Sarnoff is also developing microfluidics for applications in disparate fields, such as energy.

Orchid Changes Direction to Meet Market Demand

Orchid remained flexible in seeking ways to commercialize miniaturized, automated DNA analysis technology by acquiring promising companies and their technologies. Because the supporting technologies

were not yet available to commercialize MicroLab, Orchid used the core technical skills and knowledge from this project to move in another direction. In 1998, Orchid purchased Molecular Tool, a small Maryland company that was developing automated DNA testing based on single nucleotide polymorphisms (SNPs) (under a separate ATP-funded project, 94-05-0035, "Integrated Microfabricated Devices for DNA Typing").

Sarnoff researchers still predict that DNA analysis tools like MicroLab will move to hospitals and doctors' offices to facilitate fast, accurate diagnoses.

This technology relies on SNPs (or "snips"), which are places in the genetic code where DNA differs from one person to the next by a single letter. The SNP-based DNA analysis technology has been extremely successful in forensics and paternity testing applications. By the end of 2000, Orchid was analyzing a million SNPs per day, searching for clinically important relationships between SNPs and pharmaceuticals in attempts to find improved drug treatment options. The company completed an initial public offering in 2000 for $48 million, changed its name to Orchid BioSciences, and acquired three additional companies (GeneScreen, Inc. in 2000, Lifecodes Corp. in 2001, and Cellmark Diagnostics in 2001). Orchid changed its business model from a research and technology focus to SNP-based DNA analysis services. The company sold its research and development division to Beckman Coulter (Fullerton, CA) in 2002 and sold more than $49 million worth of DNA analyses in 2003, primarily for forensics and paternity testing.

Conclusion

The ATP-funded MicroLab research project was Sarnoff Corporation's first foray into biotechnology. Without ATP funding, Sarnoff researchers could not have developed their proposed technology beyond their initial concepts. Sarnoff developed a prototype MicroLab, received 12 patents for their microfluidics advances (microfluidics is the study of fluid behavior at tiny volumes, thousands of times smaller than a common droplet), and published their results. The company accomplished 90 percent of its technical goals. Sarnoff transferred its technology developments to its start-up company, Orchid BioComputer, beginning in 1995 and continuing through 1998 (the company was renamed Orchid BioSciences in 2000). After the project ended, Orchid acquired four other companies (Molecular Tool in 1998, GeneScreen, Inc. in 2000, and Lifecodes Corp. and Cellmark Diagnostics in 2001) and changed its business model. Orchid moved away from the original MicroLab concept and research focus. Instead, Orchid expanded paternity and forensics analyses in response to market demand, relying on technology acquired from Molecular Tool. Sarnoff continued microfluidics development and completed a feasibility study in 2003 on microfluidics' use in glucose-monitoring instruments for diabetic patients. They also started a new spinoff company based on this technology, Rosedale Medical, which developed a commercialized product the same year. Sarnoff continues to explore additional applications.

PROJECT HIGHLIGHTS
Sarnoff Corporation

Project Title: MicroLab: a High-Throughput, Low-Cost Approach to DNA Diagnostics (MicroLab: a High-Throughput, Low-Cost Approach to DNA Diagnostics by Array Hybridization)

Project: To develop a prototype, fully automated DNA "MicroLab," an instrument with miniaturized devices capable of assaying small, clinical samples of blood for a large selection of infectious, noninfectious, and genetic diseases.

Duration: 2/1/1995–1/31/1998
ATP Number: 94-05-0029

Funding (in thousands):

ATP Final Cost	$1,980	43%
Participant Final Cost	2,676	57%
Total	$4,656	

Accomplishments: Sarnoff Corporation researchers focused on the fundamentals of miniaturizing and automating DNA analysis tools. With ATP funding, they achieved 90 percent of their technical goals by developing a prototype MicroLab with the following dramatic analysis improvements:

- Reduced the sample and reagent volumes from 100 microliters to 1 microliter
- Reduced the total analysis time required from 24 hours to 3 hours
- Reduced DNA amplification and detection time from 90 minutes to 20 minutes

In 2003, Sarnoff researchers completed a feasibility study in microfluidics to develop glucose-monitoring instruments for diabetics. Sarnoff is also researching microfluidics for energy applications.

Sarnoff filed 15 patent applications from this ATP-funded technology, with the following12 patents awarded:

- "Nuclease protection assays" (No. 5,770,370: filed June 14, 1996; granted June 23, 1998)
- "Automated nucleic acid isolation" (No. 5,863,801: filed June 14, 1996; granted January 26, 1999)
- "Padlock probe detection" (No. 5,912,124: filed June 14, 1996; granted June 15, 1999)
- "Method for amplifying a polynucleotide" (No. 5,914,229: filed June 14, 1996; granted June 22, 1999)
- "Microfluidic method for nucleic acid amplification" (No. 5,939,291: filed June 14, 1996; granted August 17, 1999)
- "Assay system and method for conducting assays" (No. 5,882,903: filed November 1, 1996; granted March 16, 1999)
- "Parallel reaction cassette and associated devices" (No. 5,863,502: filed January 23, 1997; granted January 26, 1999)
- "Method for capturing a nucleic acid" (No. 5,939,261: filed June 24, 1997; granted August 17, 1999)
- "Capacitive denaturation of nucleic acid" (No. 5,993,611: filed September 24, 1997; granted November 30, 1999)
- "Sequential step method for sequencing and identifying polynucleotides" (No. 5,908,755: filed October 15, 1997; granted June 1, 1999)
- "Method for enhancing fluorescence" (No. 6,118,126: filed November 6, 1998; granted September 12, 2000)
- "Automated sample processor" (No. 6,387,710: filed November 3, 1999; granted May 14, 2002)

Commercialization Status: Sarnoff's knowledge and expertise in microfluidics have grown as a result of the technical success of this project. The MicroLab was Sarnoff's first venture into biotechnology and would not have been attempted without ATP support. Although the MicroLab itself was not commercialized, the basic concepts from this project led to the formation of Orchid BioSciences, which had more than $49 million in DNA analysis sales in 2003, primarily for forensics and paternity testing. Sarnoff researchers created another start-up company, Rosedale Medical Inc., in 2003 to pursue microfluidics applications in glucose-monitoring instruments for diabetic patients.

PROJECT HIGHLIGHTS
Sarnoff Corporation

Outlook: The outlook for microfluidic applications is good but clouded. Long-term potential medical and commercial applications are promising, although the short-term commercialization has not materialized, because the U.S. economic market has been weaker than anticipated. It has been difficult to find customers for miniaturized DNA diagnostics tools, because doctors and health insurance companies hesitate to pay for them. As consumer understanding of the benefits of DNA analyses for diagnosis grows, demand should overcome funding barriers.

Composite Performance Score: * * *

Focused Program: Tools for DNA Diagnostics, 1994

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Subcontractors:

- Dynal Biotech (formerly Dynal Direct)
  Brown Deer, WI
- Nalge Nunc International
  Rochester, NY
- Pall Corporation
  East Hills, NY
- HEI, Inc. (formerly Colorado MEDtech, Rela Inc.)
  Victoria, MN

Publications: Sarnoff researchers shared knowledge through the following publications:

- Baum, H.J., Q. Dong, P. Buffolino, R. Shaler, and Z. Loewy. "High Throughput Extraction and Stability of DNA for Genetic Diagnostics Using PCR." *American Journal of Human Genetics*. 61 (4), p. A230, Suppl. S, 1997.
- Loewy, Z.G., Q. Dong, P. Buffolino, R. Shaler, and H.J. Baum. "Correlation of Sample Preparation Methodology with Efficiency of PCR." *Clinical Chemistry*. 43 (11), p. 29, 1997.

Presentations: Sarnoff researchers also disseminated their findings through the following presentations:

- Cherukuri, Satyam. "Microarray Challenges: Going Forward." Biochip Array Technologies Fabrication and Applications Conference, Washington, DC, May 1995.
- "Nucleic Acid Sample Processing." DNA Probes Conference, San Diego, CA, 1997.